Effect of Prior Exposure to Hydrostatic Pressure on Rainbow Trout (*Oncorhynchus mykiss*) Survival in Air-Supersaturated Water

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EFFECT OF PRIOR EXPOSURE TO HYDROSTATIC PRESSURE ON RAINBOW TROUT (*Oncorhynchus mykiss*) SURVIVAL IN AIR-SUPERSATURATED WATER

by

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ABSTRACT

Antcliffe, B.L., L.E. Fidler, and I.K. Birtwell. 2003. Effect of prior exposure to hydrostatic pressure on rainbow trout (*Oncorhynchus mykiss*) survival in air-supersaturated water. Can. Tech. Rep. Fish. Aquat. Sci. 2501: 11p.

We examined the effect of prior exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to hydrostatic pressure (water depth) on their survival during subsequent exposure to air-supersaturated water, measured as Total Gas Pressure (TGP). Our hypothesis was that hydrostatic pressure would reduce nucleation sites within the cardiovascular system from which gas emboli originate, thereby extending time to mortality in air-supersaturated water. During acclimation (4 h), one group of fish received the hydrostatic pressure treatment at 2.5 m depth while corresponding fish not pre-exposed to hydrostatic pressure were confined to the surface. All fish were subsequently exposed to 122% TGP, 10°C in 0.25 m for 48 h. Separate control fish held in air-equilibrated water survived. Survival in air-supersaturated water was marginally higher for fish pre-exposed to hydrostatic pressure; however, the effect of prior hydrostatic pressure on survival was not significant after 18, 24, 36 and 48 h of exposure. Therefore, fish use of deep water before encountering lethal TGP in shallow water did not improve survival markedly. Results have implications for determining TGP exposure and biological effects.

RÉSUMÉ

Antcliffe, B.L., L.E. Fidler, and I.K. Birtwell. 2003. Effect of prior exposure to hydrostatic pressure on rainbow trout (*Oncorhynchus mykiss*) survival in air-supersaturated water. Can. Tech. Rep. Fish. Aquat. Sci. 2501: 11p.

Nous avons examiné l'effet d'une exposition préalable de juvéniles de truite arc-en-ciel (*Oncorhynchus mykiss*) à la pression hydrostatique (profondeur de l'eau) sur leur survie pendant une exposition subséquente à de l'eau sursaturée en air, dans laquelle on mesure la pression totale du mélange gazeux (TGP). Nous avions pour hypothèse que la pression hydrostatique allait réduire les sites de nucléation, dans le système cardiovasculaire, d'où proviennent les embolies gazeuses, ce qui permettrait d'étendre le délai jusqu'à la mortalité dans l'eau sursaturée d'air. Pendant l'accoutumance (4 h), un groupe de poissons a été soumis à un traitement de pression hydrostatique correspondant à 2,5 m, tandis que leurs congénères qui n'avaient pas été exposés préalablement à cette pression hydrostatique étaient confinés en surface. Tous les poissons ont ensuite été exposés à une TGP de 122 %, à 10 °C, dans 0,25 m d'eau, pendant 48 h. Des poissons témoins maintenus séparément dans une eau à l'état d'équilibre gazeux ont survécu. La survie dans l'eau sursaturée en air était marginalement plus élevée pour les poissons préalablement exposés à la pression hydrostatique; toutefois, cet effet n'était pas significatif après 18, 24, 36 et 48 h d'exposition. Le fait que les poissons s'étaient tenus en eau profonde avant d'affronter une TGP létale en eau peu profonde n'a donc pas amélioré la survie de façon marquée. Ces résultats ont des incidences sur les mesures de l'exposition à la TGP et des effets biologiques.

INTRODUCTION

Water is air-supersaturated when the partial pressures of atmospheric gases in solution exceed their respective partial pressures in the atmosphere. It is commonly referred to as dissolved gas supersaturation (DGS), and is often expressed in terms of Total Gas Pressure (TGP) and/or ΔP (the pressure differential between dissolved gas pressure and interfacial gas phase pressure). DGS can lead to a physiological condition in fish known as gas bubble disease, which can be harmful or even lethal due to the formation of gas emboli within the cardiovascular system, causing blockage of blood flow and death by hemostasis (see Stroud and Nebeker 1976; Bouck 1980). Sources of DGS include primary production, solar heating, water falls, and release of water over spill structures at hydroelectric facilities.

Although the effects of DGS on fish and other aquatic biota have been studied since the early 1960s (see review by Weitkamp and Katz 1980), there remains considerable controversy regarding safe TGP levels for wild fish populations. This controversy arises in part because most of the literature data were developed by static laboratory bioassays using constant TGP and shallow depths. However, in the wild, fish experience dynamic exposure conditions caused by spatial or temporal changes in TGP and fish movement. Vertical movement is a critical component of the TGP exposure because as a fish descends into deeper water TGP remains constant (in a well-mixed water column), but hydrostatic pressure increases. Correspondingly, there is a reduction in ΔP. Depending on the depth, this may prevent formation of gas emboli in the cardiovascular system or cause resolubilization of emboli that formed at lower hydrostatic pressures (Knittel et al. 1980; Fidler 1988). The depth at which the hydrostatic pressure negates the adverse effect of the TGP level is termed the TGP compensation depth (i.e., $\Delta P = 0$), and it is the depth at or below which gas bubbles cannot form in fish (Bouck 1980).

Previous studies have shown that fish use of greater water depths during exposure to elevated TGP, or intermittent use of shallow and deeper water, increased their survival relative to that for a shallow water exposure alone during exposure to lethal TGP (e.g., Ebel 1971; Weitkamp 1976; Knittel et al. 1980; Antcliffe et al. 2002). There is, however, little information to ascertain whether the prior use of deeper water by fish before encountering lethal TGP in shallow water has a beneficial effect on survival. Although the results of Aspen Applied Sciences Incorporated (1998) suggest that there is a beneficial effect, their experiment consisted of only one replicate of 30 fish per treatment group, and the fish were exposed to very high TGP of 140% in shallow water. If this effect is real, then the previous hydrostatic pressure history of the fish before exposure to elevated TGP would be a critical component of the dynamic exposure, along with use of water depth during the exposure, for assessing the biological effects of air-supersaturated water on wild fish populations.

Our purpose was to examine the effect of prior exposure of juvenile rainbow trout to hydrostatic pressure (water depth) on survival during subsequent exposure to lethal DGS in shallow water. Our hypothesis was that the hydrostatic pressure would reduce the size of pre-existing nucleation sites within the cardiovascular system from which gas emboli originate; thereby, extending the time to mortality over the exposure period. That is, it would take longer for the smaller nucleation sites to grow into macroscopic bubbles within the cardiovascular

system, where they can continue to grow and accumulate, eventually causing blockage of blood flow and death.

METHODS

MATERIALS AND APPARATUS

Juvenile rainbow trout (*Oncorhynchus mykiss*) were transported from the Vancouver Island Trout Hatchery to the nearby Rosewall Creek Hatchery in BC, Canada, in an insulated tank supplied with compressed air from a portable compressor. The fish were reared in a 2,700-L circular fibreglass tank with air-equilibrated groundwater heated electrically to 10°C, at a flow rate of 25 $L \cdot min^{-1}$ and depth of 0.75 m. Fish were fed a maintenance ration of 2.5% body weight per day of commercial feed delivered uniformly between dawn and dusk using two automatic timers to minimize variability in fish size due to the establishment of dominance hierarchies. Fish were held at Rosewall for three months prior to experimentation in May 2001.

The air-equilibrated or control water that supplied the stock tank, laboratory deep tank, and experimental control tanks flowed to an aeration tower to remove excess DGS that developed during heating. This produced approximately 101 % TGP at 10°C. Treatment water was air-supersaturated to 122% TGP at 10°C by exposing it to medical grade air within a pressurized PVC column (0.2 m diameter by 2.5 m height), packed to a depth of 2 m with bio-rings that acted as mass transfer media. The operating pressure of the column was fixed by water pressure and TGP was controlled by adjusting the water level and air-flow rate using an automatic level control system. Air-supersaturated water flowed by gravity into the experimental treatment tanks, and a small flow (3 $L \cdot min^{-1}$) was diverted to a tall PVC column (10 cm diameter by 3.6 m height) to allow for accurate measurement of TGP under hydrostatic pressure.

Fish received the hydrostatic pressure treatment in an opaque, cylindrical, 900 L fibreglass laboratory deep tank (0.6 m diameter by 3 m height) supplied with a flow rate of 15 $L \cdot min^{-1}$. Fish were confined to depth by holding them in cylindrical cages (0.29 m diameter and 0.25 m deep) constructed from Vexor™ (plastic) mesh. Fish that did not receive the hydrostatic pressure treatment were held at the surface (to a depth of 0.25 m) in a flat-bottomed Vexor™ mesh liner.

Fish were exposed to air-supersaturated water in 25 L cylindrical fiberglass tanks (0.36 m diameter by 0.45 m height). Ten pairs of tanks were aligned in series such that one row of tanks received control or air-equilibrated water while the other received air-supersaturated water. This allowed for 8 replicates, each consisting of a control and treatment tank, to be tested simultaneously, leaving one set of tanks for measurement of water quality and a final set was not used (to accommodate even split between the two treatments of prior exposure to hydrostatic pressure versus none). The control and air-supersaturated water flowed from separate header lines into the experimental tanks via a valve fitted with clear plastic tubing and PVC pipe (approximately one half each). The outlet end of the PVC pipe was attached to the bottom of each tank and it was fitted with a flow restriction nozzle to control flow rate and prevent pressure losses and off-gassing along the distribution system. A surface outflow also prevented degassing. The flow rate to all experimental tanks was 2.4 $L \cdot min^{-1}$. Crossing the water delivery hoses every other pair of tanks varied the location of the control and treatment tanks, and this

ensured that any external factors, such as movement, noise, or lighting, affected control and treatment tanks equally. All tanks were covered with a black circular lid that contained a small viewing window covered with red cellophane to reduce disturbance from background lighting and human observers. Overhead lighting simulated the natural photoperiod.

The flow rates to the stock, deep, and experimental tanks all provided 90% water replacement times that ranged from 24 min to 4 h, which were below the 4.5 to 7 h limit recommended by Sprague (1973) for pollutant bioassays. In all cases, fish density and flow loading were below the limits in Sprague (1973) of 10 $kg \cdot m^{-3}$ and 0.5 to 0.7 $kg \cdot L^{-1} \cdot min^{-1}$, respectively.

PROCEDURES

The experiment consisted of a 4 h acclimation period where the two treatments were applied. That is, one group of fish received the hydrostatic pressure treatment of 2.5 to 2.75 m water depth in a laboratory deep tank, while the corresponding group of fish that did not receive the hydrostatic pressure treatment were confined to the water surface. All fish were then randomly assigned to the experimental treatment tanks for subsequent exposure to 122% TGP at 10°C in 0.25 m depth for 48 h. Control fish (held in air-equilibrated water to control for mortality from sources other than air-supersaturation) were randomly allocated to the experimental control tanks for the 4 h acclimation and 48 h exposure period. Each experimental tank contained 20 fish. The experimental procedure was repeated twice, subsequently and under identical conditions, to produce 16 replicates, or a total of 320 control fish and 320 treatment fish exposed to 122% TGP (160 for each of the two treatments).

Several hundred fish were transferred from the stock tank to a smaller holding tank at once using a large net. Three groups of 28 fish were selected by dip-net and placed into separate 13 L plastic buckets. These fish were randomly allocated to the three cages suspended at the surface of the deep tank. The cages were slowly lowered in unison to 2.5 to 2.75 m depth for the top and bottom of the cage, respectively. Three groups of 28 fish were placed into the surface liner at the top of the deep tank. Eight groups of 20 fish were then randomly assigned to the control tanks.

After acclimation, fish at the surface of the deep tank were transferred in four batches of 20 fish per 13 L bucket to four randomly selected experimental treatment tanks. Excess fish were discarded. The cages in the deep tank were slowly raised to the surface together, at a rate of 1 m per 10 s. These fish were transferred, also in four batches of 20 fish per 13 L bucket, to the remaining randomly allocated treatment tanks. Since all treatment tanks contained air-supersaturated water at 122% TGP, the buckets were initially set beside each experimental tank until all were in place; they were then emptied in random order all within 2 minutes. Buckets were covered with a dark lid during transport.

Fish were not fed during the experiment and they were observed after 6 h, 12 h, 18 h, 24 h, 36 h, and 48 h of exposure to 122% TGP. Fish were declared dead following loss of equilibrium and cessation of opercula movement. When dead fish were removed from a treatment tank the corresponding control tank was netted to provide the same disturbance. Dead fish were blotted dry, weighed, and their fork length was determined. Fish that survived were euthanized using a

lethal (200 $mg \cdot L^{-1}$) dose of buffered (pH = 7.0) tricaine methanesulphate (MS-222), and measured for length and weight.

TGP of the air-supersaturated water was measured every 30 min using two total dissolved gas meters (Common Sensing Inc., Clark Fork, Idaho; accuracy ± 0.2 mm Hg) equipped with data logging capabilities. Results of both meters, which were similar, were averaged. A tensionometer (Alpha Designs Model 300C, Victoria, BC; accuracy ± 0.2 mm Hg) measured TGP of the air-equilibrated water. The calculations in Colt (1984) were applied to derive the specific dissolved gas parameters. Temperature was recorded every 10 min using Onset Stowaway Tidbit loggers (accuracy ± 0.2°C).

STATISTICAL ANALYSIS

The Cochran Mantel Haenszel (CMH) statistic was used to analyse the data using cumulative mortality at time 24, 36, and 48 h as the response variable. The CMH test is an extension of the chi-square test for contingency tables applicable when the study design contains a blocking factor (e.g., replicates). The CMH statistic tested the null hypothesis that there was no association between fish state (dead or alive) and the treatment (prior exposure to hydrostatic pressure versus none), after controlling for the effect of replicates in a multi-way frequency table. Fish weight and length were compared using *t* tests and the non-parametric equivalent (Kruskal-Wallis) because in a few cases the assumption of normality was not met. In all cases the non-parametric test confirmed results of the parametric *t* test, and *p* values for the *t* tests were reported.

RESULTS

TGP and temperature were maintained within a narrow range during experimentation. Mean (± SD) TGP for treatment and control water were 121.9 ± 0.5% (ΔP = 167 ± 4 mm Hg) (n = 97) and 101.5 ± 0.5% (ΔP = 13 ± 4 mm Hg) (n = 24), respectively. Maximum and minimum values were 122.9% and 120.9% TGP for air-supersaturated water and 101.2% and 102.5% TGP for control water. The O_2/N_2 ratio in percent saturation ranged from 0.97 to 0.96 for treatment water and 0.99 to 1.01 for control water. Temperature averaged 10.1 ± 0.2°C for treatment and 10.2 ± 0.2°C for control tanks (n = 289).

All control fish survived. All treatment fish survived the first 12 h of exposure to 122% TGP. The initiation of mortality (i.e., time to death of the first fish) was similar for both treatments, in that the initiation of mortality for each treatment was from 12 to 18 h for 4 of the 8 replicates and from 18 to 24 h for the remainder.

Mean percent cumulative mortality was higher for fish that did not receive the hydrostatic pressure treatment (i.e., fish acclimated at the water surface) compared to that for fish that had prior exposure to hydrostatic pressure (i.e., fish acclimated at depth) after 18, 24, 36, and 48 h of exposure to 122% TGP. However, these differences were most pronounced after 48 h of exposure (Fig. 1), and percent cumulative mortality did not differ significantly between the two treatments after 24 h (p=0.870), 36 h (p=0.579), and 48 h (p=0.261) of exposure to 122% TGP. That is, there was no association between fish state (dead or alive) and the treatment. The odds

ratio of 0.775 (calculated from the CMH test) suggested that there was a slightly higher chance of death when fish were acclimated at the surface compared to depth, but the 95% confidence interval for the odds ratio contained the number 1.0, indicating there was no association between fish state and the treatment. The Breslow-Day test for homogeneity of odds ratios (p=0.580) also showed no evidence against homogeneity in odds ratios across the frequency tables.

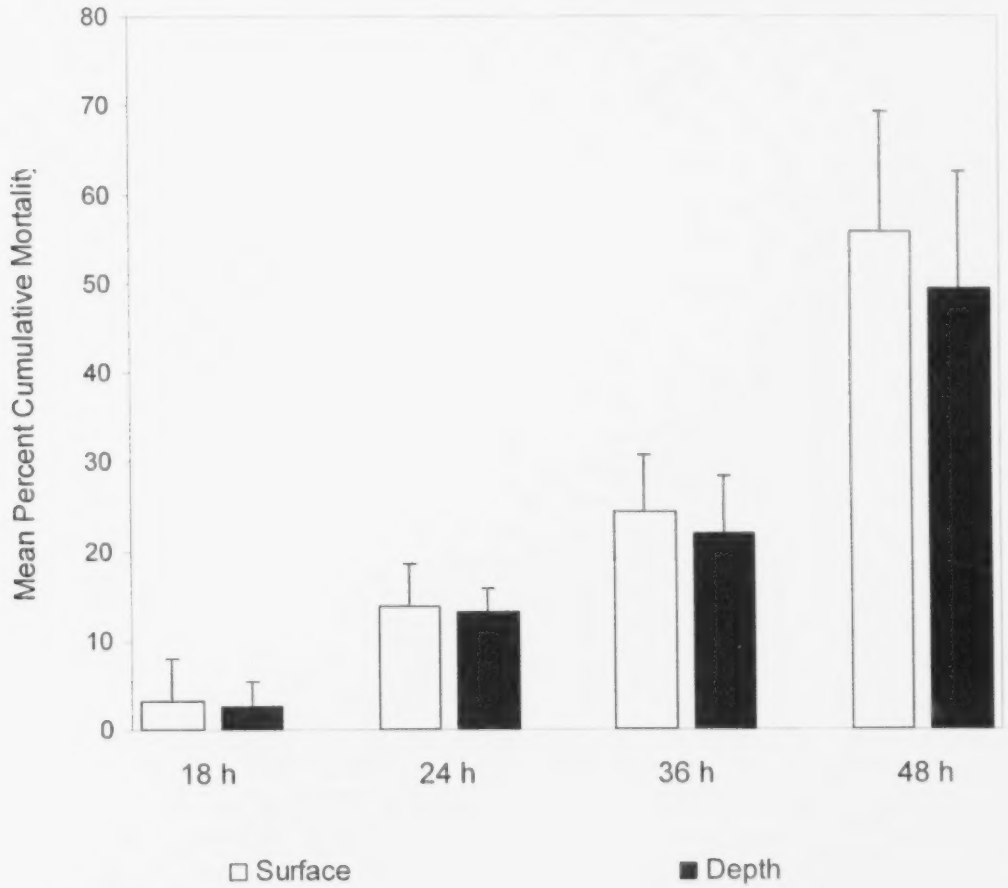

Figure 1. Mean (± SD) percent cumulative mortality for juvenile rainbow trout exposed to 122% TGP at 10°C in 0.25 m after 18, 24, 36, and 48 h for fish previously exposed to hydrostatic pressure of 2.5 to 2.75 m water depth (acclimated at "Depth") and fish that had no prior exposure to hydrostatic pressure (acclimated at the "Surface"), during a 4 h acclimation period in air-equilibrated water. Percent cumulative mortality was zero after 12 h of exposure for all replicates. All control fish (held at 101% TGP) survived.

There was little variation in fork length or weight (mean ± SD) of control (96.7 ± 9 mm; 10.2 ± 3 g) and treatment (95.4 ± 9 mm; 9.8 ± 2 g) fish, and differences in length (p=0.314) and weight (p=0.266) were not significant. There were no significant differences in length (p=0.287)

or weight (p=0.318) between treatment fish that had prior exposure to hydrostatic pressure (94.9 ± 9 mm; 9.6 ± 2 g) and those that did not (95.9 ± 8 mm; 9.9 ± 3 g). Further, there were no significant differences in length (p=0.413) or weight (p=0.486) between fish that died and those that survived the exposure to 122% TGP.

DISCUSSION

Previous studies by Aspen Applied Sciences Incorporated (1998) indicated that fish exposed to hydrostatic pressure prior to encountering lethal TGP in shallow water had an increased resistance to mortality. They showed that the time to initiation of mortality for chinook salmon exposed to 140% TGP at 10°C near the water surface was more than twice as long for fish that received the hydrostatic pressure treatment (3 m of water depth at 100% TGP for 3 h), compared to that for fish without prior exposure to hydrostatic pressure. Fish that had no prior exposure to hydrostatic pressure started to die after 3 h, while fish that received the hydrostatic pressure treatment began to die after 6.7 h. They also showed that the time to mortality for the other fish that died later and had received the prior exposure to hydrostatic pressure was longer than that of the fish that had not received the hydrostatic pressure treatment.

The results of Aspen Applied Sciences Incorporated (1998) and the earlier work of Fidler (1988) formed the basis for our hypothesis that hydrostatic pressure would reduce the size of pre-existing nucleation sites within the cardiovascular system from which gas emboli originate. As reviewed by Fidler (1988), the appearance of macroscopic bubbles in physical or physiological systems is dependent on pre-existing gas-filled microscopic nucleation sites. Normally, nucleation sites are stabilized, in part, by a combination of the liquid cohesive forces, surface tension forces, and the adhesive forces between the liquid and the surface to which the nucleation site is attached. Philip et al. (1972) also showed that in blood, platelets and other protein components aggregate at the nucleation site air – blood interface, which may provide other stabilization mechanisms. The remaining forces are those associated with the internal pressure in the nucleation site and the liquid dissolved gas pressure. If any of these forces or pressures change, the nucleation site will either shrink or grow, depending on the nature of the change. When hydrostatic pressure increases while all other forces remain constant, the internal pressure in the nucleation site will increase. Gas will then diffuse out of the nucleation site and it will decrease in size until a new balance of forces is reached. If fish containing smaller nucleation sites are brought to the surface from depth, it may take longer for the nucleation sites to grow into macroscopic bubbles in the cardiovascular system; thereby, extending the time to mortality.

Our results differed from those of Aspen Applied Sciences Incorporated (1998) in that there was no significant effect of the 4 h hydrostatic pressure treatment on percent cumulative mortality of juvenile rainbow trout exposed to 122% TGP over a 48 h period in shallow water. Although the trend was for higher survival for fish that received the hydrostatic pressure pre-treatment, differences were most pronounced only after 48 h of exposure, during which 50% or more of the fish from both treatments died. After 36 h, there was little difference in percent cumulative mortality between the two treatments (i.e., 22% for fish that had prior exposure to hydrostatic pressure compared to 24% for fish that did not). Our results also indicated that there was no effect of the prior exposure to hydrostatic pressure on the initiation of mortality.

A key difference between our experiments and those of Aspen Applied Sciences Incorporated (1998) was the TGP level. As a result, the time required to initiate mortality at the lower TGP of 122% used in our studies was considerably longer (approximately 18 h on average) than that required in the studies at 140% TGP (approximately 3 to 6.7 h). If existing nucleation sites were reduced in size during the prior exposure to hydrostatic pressure, then the nuclei could have re-grown to near their original size soon after the fish were placed in shallow water for their exposure to 122% TGP. Since the hydrostatic pressure was similar for both experiments, the time required for the nuclei to re-grow to their original size in shallow water would presumably be a smaller percent of the total time required to initiate mortality at 122% TGP, compared to 140% TGP. It would then be expected that the effect of prior exposure to hydrostatic pressure would be most pronounced at high TGP (e.g., 140%), which is consistent with our results and those of Aspen Applied Sciences Incorporated (1998). Our studies used 122% TGP because it is relevant for local water bodies.

Fish species also differed between our experiments and those of Aspen Applied Sciences Incorporated (1998). Although the effect of hydrostatic pressure on the susceptibility of various fish species to DGS is unknown and could not be determined from our experiments, our studies used rainbow trout because they have been shown to be more susceptible to mortality from DGS than chinook salmon (see Rucker 1975; Nebeker and Brett 1976) and they are ubiquitous in local river systems. The fish used in our experiments (91 to 97 mm fork length) were also smaller than those used in Aspen Applied Sciences Incorporated (1998) (139 to 154 mm fork length), however, based on studies by Jensen et al. (1986), we would expect only minor differences if any in susceptibility to air-supersaturated water for fish in this size range.

Previous studies on the beneficial effect of fish use of water depth on survival in air-supersaturated water (e.g., Antcliffe et al. 2002) were unable to examine the effect of hydrostatic pressure on pre-existing nucleation sites prior to the formation of gas emboli within the cardiovascular system because the fish were exposed to lethal TGP in shallow water above the compensation depth before they experienced greater hydrostatic pressure at depth. In our experiments the fish were not exposed to elevated TGP before they received the hydrostatic pressure treatment. Thus, we examined the direct effect of hydrostatic pressure prior to exposure to elevated TGP, or presumably on pre-existing nucleation sites from which gas emboli originate in the cardiovascular system.

Although our experiments were limited to a hydrostatic pressure of 2.5 to 2.75 m, greater water depths would be expected to reduce nucleation sites even further, and perhaps further delay their re-growth and the onset to mortality for any given TGP in shallow water above the TGP compensation depth. This would be applicable in rivers such as the Columbia, where fish could be exposed to hydrostatic pressures of 30 meters or more.

Our results have implications for determining TGP exposure components and assessing the biological effects of air-supersaturated water on wild fish populations, where dynamic exposure conditions prevail due to fish movement, migration or behavioural patterns that result in use of different water depths (e.g., diel vertical migration, use of shallow water margins for feeding, escape to cover in deep-water habitat for predator avoidance). They suggest that, in the range tested, fish use of water depths below the TGP compensation depth prior to encountering lethal

TGP in shallow water will not improve survival markedly. In contrast, others have found that fish use of deeper water during exposure to TGP will increase survival significantly, relative to that for a shallow-water exposure alone. In conclusion, for our experiment, the TGP exposure components consisted of the TGP level and fish use of water depth during the exposure to TGP, but not the prior hydrostatic pressure history of the fish before exposure to the TGP.

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